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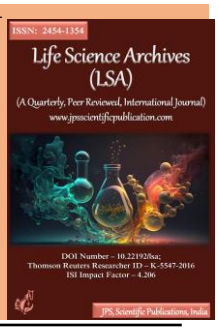

Research Article

DETECTION OF *Bla VIM-2* GENE IN *Pseudomonas aeruginosa* ISOLATED FROM DIFFERENT SOURCES IN HILLA PROVINCE

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Abstract

Pseudomonas aeruginosa carrying the *Bla VIM-2* gene has been reported worldwide is known as one of the prevalent types of metallo β lactamase ranking second only to NDM. These genes can be horizontally. Are often associated with Integron gene cassettes. This research paper explores the investigation. The study focused on examining the presence of *Bla VIM-2* in strains of *Pseudomonas aeruginosa*. Between November 2022 and February 2023, a total of one hundred patients were admitted to Hilla Teaching Hospitals, in Babylon province. A diverse set of 100 samples was collected for analysis including 50 burn swabs (50 %), 10 ear swabs (10 %), 18 wound swabs (18 %), 3 sputum samples from patients with tract infections (3.5 %) and an additional 19 wound swabs (19 %). The identification of carbapenemase genes was carried out using the Polymerase Chain Reaction (PCR) technique. The findings revealed that among the three *Pseudomonas aeruginosa* samples to carbapenems, 43 (6.97 %) harbored the *Bla VIM-2* gene.

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1. Introduction

Indian cuisine has a very prominent position among all the other cuisines. *Pseudomonas aeruginosa*, a bacterium with a rod-shaped structure that does not ferment and is classified as Gram-negative, has emerged as a major issue in infections acquired in hospitals, particularly affecting patients with weakened immune systems. The prevalence of *Pseudomonas aeruginosa* infection is increasing globally as a result of its ability to survive, adapt and develop resistance to various antimicrobial agents (Moradali *et al.*, 2017). The inherent resistance of *Pseudomonas aeruginosa* and its capacity to develop resistance to many antibiotics at some stage in treatment drastically restricts the range of powerful therapeutic choices. Hence, it's far important to possess information regarding the resistance patterns frequent in the neighborhood place as a way to decide the maximum appropriate remedy techniques (Morrissey *et al.*, 2013; Weiner *et al.*, 2016).

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Pseudomonas aeruginosa reveals many antibiotic resistance pathways, which may be categorised as intrinsic, received, and adaptive (Gales *et al*., 2012). Acquired resistance can get up from mutations or the purchase of outside resistance factors. It may be facilitated with the aid of diverse methods, including enzyme degradation, reduced permeability, and energetic efflux (Hidalgo *et al.,* 2016). Intrinsic resistance is received by way of inherent structural or functional traits, which includes having a low permeability within the outer membrane, the potential to expel antimicrobials, and the creation of enzymes that render antibiotics ineffective (Pang *et al*., 2019). Adaptive resistance affects the lungs of patients *via* developing biofilms that act as a barrier in opposition to the penetration of antimicrobial agents (Tsakris *et al*., 2000; Rossolini and Mantengoli, 2005).

Carbapenems are a set of β-lactam antibiotics that have a large spectrum of antibacterial outcomes. β-lactamases possess a sturdy resistance because of their precise structure. Meropenem and imipenem are both effective in treating infections resulting from *Pseudomonas aeruginosa*, and they are a number of the few remedy options available (Sader *et al*., 2015). Carbapenems are largely considered the maximum desired antibacterial medicines and are often used to deal with tough *Pseudomonas aeruginosa* infections. The international incidence of carbapenem resistance in *Pseudomonas aeruginosa* has been gradually increasing. Nevertheless, the precise effect of various reasons of carbapenem resistance stays uncertain (Gad *et al*., 2007; Magliano *et al*., 2012; Al-Huraishi, 2016).

The aim of this present investigation was to detect the presence of *Bla-VIM 2* producers in *Pseudomonas aeruginosa* isolates collected from clinical samples received from hospitals in Hilla. Furthermore, analyze the associations between the presence of resistance genes *Bla-VIM 2* and the vulnerability to 10 antibiotics.

2. Material and Methods

Samples Collection

Samples collection from 200 patients who were admitted to Mejan Teaching Hospital and Al-Hilla Teaching Hospital in Hilla province, Iraq in duration between November 2022 and February 2023. The samples comprised burn swabs, ear swabs, urine, sputum, and wound swabs.

Identification of *Pseudomonas aeruginosa*

Two swabs were obtained from each subject for the collection of swab samples. Regarding the sputum and urine, they were separated into two portions. The initial swab was utilized for creating a stained smear for the purpose of Gram staining, however the second swab was employed for cultivating on different culture media. This facilitated the separation and identification of the factors responsible for causing the observed effects. *Pseudomonas aeruginosa* isolates were cultivated in Brain-Heart Infusion (BHI) broth with the addition of 15 % glycerol, and the tubes were stored at a temperature of -20 °C (Villegas *et al*., 2007).

Antibiotics susceptibility test using Disk diffusion technique

The antibiotic resistance profiles of the *Pseudomonas aeruginosa* isolates were evaluated using the Kirby-Bauer disk diffusion test on Muller Hinton agar media.

DNA Extraction and PCR procedure

This task entailed the retrieval of both plasmid DNA and chromosomal DNA. The plasmid DNA extraction was conducted following the methodology outlined in reference (Poirel *et al*., 2010). The carbapenem-resistant isolates underwent regular PCR testing using specific primers designed for the detection of the blaNDM-1 gene (F- TCTACATGACCGCGTCTGTC and R- TGTGCTTTGACAACGTTCGC) (Yong *et al*., 2009).

3. Results and Discussion

This experiment involved the collection of 100 samples. These samples included burn swabs (n = 50, 0.50 %), ear swabs (n = 10, 10 %), wound swabs (n = 18, 18.00 %), saliva from people who had an infection of the lower respiratory tract (n = 3, 3.50 %), and additional wound swabs (n = 19, 19 %). The age range of the patients spanned from zero to over 61 years (Table - 1). *Pseudomonas aeruginosa* has exhibited extensive antibiotic resistance, as indicated in the Table – 2 below.

Table – 1: The Age Range of Patients

Age groups	*Pseudomonas aeruginosa*	Other bacteria	Negative	Total
1 - 10 years	5	5	1	11
11 - 20 years	4	5	1	10
21 - 30 years	9	7	2	18
31 - 40 years	10	10	2	22
41 - 50 years	5	5	4	14
51 - 60 years	4	4	2	10
>61 years	6	6	3	15
Total	**43**	**42**	**15**	**100**

Table – 2: Antibiotic Resistance Exhibited by *Pseudomonas aeruginosa*

Sample type	Imipenem (10) ≤1314 -15≥ 16	Chloramphenicol (10) ≥3216≤ 8	Ciprofloxacin (10) ≥42≤ 1	Amikacin (10) ≥6432≤16	Tetracyclin (10) ≥168≤4	Rifampin (5) ≤1617 -19≥19
Burns	20	0	10	5	0	0
Wounds	40	0	40	10	0	2
Sputum	40	0	2	10	0	0
Respiratory infection	40	0	0	15	0	0
Environmental	35	0	40	0	0	0

The findings of the current investigation demonstrated the presence of a single band while amplifying the plasmid DNA of *Pseudomonas aeruginosa* using the blaNDM-1 primer. The electrophoresis analysis revealed a single band with a size of 100 base pairs (Figure - 1).

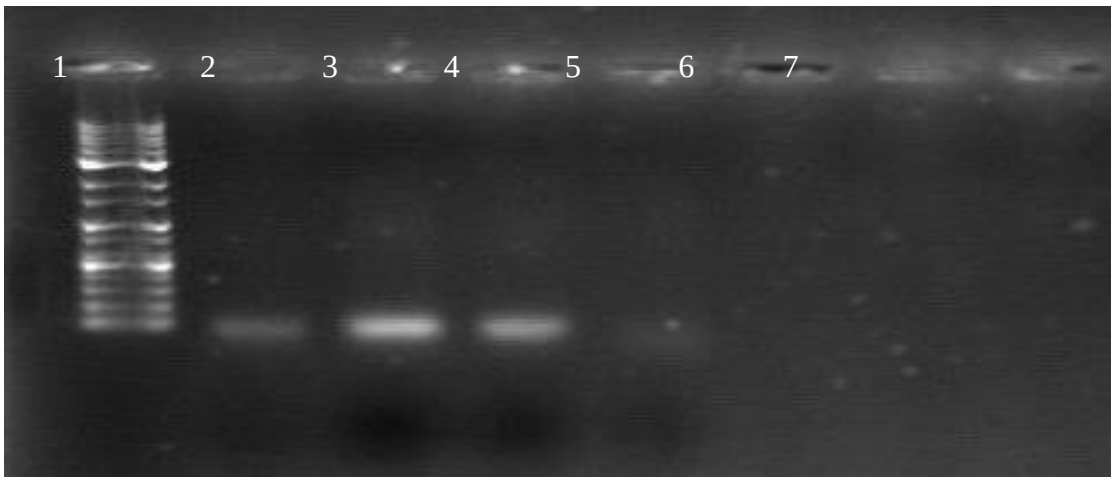

Figure - 1: The amplified plasmid DNA containing the MBL *Bla- NDM1* gene (100bp) was subjected to Gel Electrophoresis using PCR with particular Primers. The gel used was made of 1.5 % Agarose and the Electrophoresis process lasted for 90 minutes at a temperature of 70V\cm

Research has shown that the purchase of *Bla NDM* is the principle reason of the rapid look and dissemination of bacteria that generate carbapenemase on this sub-continent (Yong *et al.*, 2009). The emergence of carbapenem-resistant *Pseudomonas aeruginosa* affords a tremendous assignment to clinical strategies due to its excessive propensity for gaining resistance to many medicine classes and its inherently reduced susceptibility to numerous antimicrobials (Magliano *et al.*, 2012). The average age of the sufferers in the one hundred samples covered in this investigation become 32.44 years.

Carbapenems, a kind of β-lactam antibiotics, show off efficacy against *Pseudomonas aeruginosa* microorganism. Nevertheless, the emergence and dissemination of obtained carbapenem resistance on this specific species have posed demanding situations in phrases of treatment and contamination control (Gad *et al.*, 2007). The consequences of our take a look at contradicted the results of Gupta *et al.* (2006) who discovered that imipenem had extra efficacy than meropenem (Al-Huraishi, 2016). The preceding takes a look at mentioned resistance charges of 7.4 % and 14.8 % for imipenem and meropenem, respectively. The have a look at found out that eighty three. Totally, 50 % of the isolates exhibited resistance to fluoroquinolones, with 60.19 % demonstrating resistance to ciprofloxacin and a same percentage demonstrating resistance to Levofloxacin. The resistance costs discovered on this look at are markedly greater than the quotes suggested in a previous look at performed in Najaf Hospitals. In that observe, the resistance rates for Ciprofloxacin and Levofloxacin were 73.4 % and 55 % respectively (Riera *et al.*, 2011; Al-Kadhmi *et al.*, 2016). Moreover, it is steady with the outcomes of a preceding investigation achieved in Najaf (Gupta *et al.*, 2006).

Pseudomonas aeruginosa traces accumulated from Babylon hospitals are proven an increasing resistance to Fluoroquinolones. This trouble may be explained by the combined impact of increased fluoroquinolone usage and the hospitals' failure to comply with established infection manage practices. Unlike a previous look at conducted in Najaf (Al-Shara, 2013) which indicated a resistance rate of simplest 64.8 % for *Pseudomonas aeruginosa* isolates to this antibiotic, the current investigation found better resistance costs of eighty 5.44 % for Amikacin and ninety on, and 26 % for gentamicin for *Pseudomonas aeruginosa* isolates. Nevertheless, the consequences of the *Pseudomonas aeruginosa* antibiogram in this look at are in direct opposition to those of a examine conducted within the USA of America (Al-Muhannak, 2015). The findings of the present study reveal that 51.46 % of the *Pseudomonas aeruginosa* isolates exhibited resistance to aztreonam. The results of this investigation indicate a higher rate of aztreonam resistance among *Pseudomonas aeruginosa* clinical isolates compared to the findings of a prior study conducted by Abdullah, which revealed a lower percentage of resistance (Prickett *et al.*, 2015).

Further investigations will ascertain the direction (either from chromosome to plasmid or vice versa) of the transfer that took place within the organism. Wild strains and transformants harboring *blaVIM-2* and *blaNDM-1* displayed a broad spectrum of elevated Minimum Inhibitory Concentrations (MICs) and a reduced susceptibility to the majority of the antibiotics examined (Toleman *et al.*, 2007). Previous studies have shown that strains producing carbapenemase are still vulnerable to Polymyxin B. Nevertheless, both of our research isolates exhibited resistance to this antibiotic, so creating a challenging scenario with few or no available therapeutic alternatives. The presence of *blaVIM-2* in a mobile genetic element can enhance their ability to spread to other vulnerable organisms. After examining the transmission dynamics of the strains, it became evident that *blaVIM-2* and *blaNDM-1* have the ability to be horizontally transferred. It is important to mention that although the initial host *Pseudomonas aeruginosa* was unable to pass on its DNA to *Escherichia coli*, our investigation demonstrated the successful transfer of the plasmid encoding the *NDM-1* gene from the *Escherichia coli* mutants to the receiving

Escherichia coli strain *via* conjugation. The absence of plasmid conjugation in these two strains can be attributed to physiological hurdles, adverse laboratory conditions before the start of conjugation, or the lack of a functional *Tra* operon in the present plasmid of *Pseudomonas aeruginosa*. Serial passage of transformants containing both *blaVIM-2* and *blaNDM-1* genes demonstrated that the *blaNDM-1* gene exhibits greater stability than *blaVIM-2*, possibly due to the fact that *Escherichia coli* is not a natural host for *blaVIM-2* environment.

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